

11/28/00
1944 U.S. PTO

12 - 04 - 00

Attorney Docket No.: A-576C

DIVISION - CONTINUATION - CONTINUATION-IN-PART
APPLICATION TRANSMITTAL FORMJL682 U.S. PTO
09/28/2000
11/28/2000

	Anticipated Classification Of This Application: Class 424	Prior Application: Examiner F.T. Mozie	Art Unit 1653
--	---	--	------------------

To the Assistant Commissioner for Patents:

This is a request for filing a continuation divisional continuation-in-part application, under 37 CFR 1.53(b), of pending prior application Serial No. 09/221,181 filed on December 23, 1998 of Goldenberg, et al.

for POLYOL/OIL SUSPENSIONS FOR THE SUSTAINED RELEASE OF PROTEINS

For CONTINUATION or DIVISIONAL APPLNs only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 1b, below, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

1. Transmitted herewith are:
 - 29 pages of specification, 3 pages of claim(s) and 1 page of abstract, totaling 33 pages.
 - 3 pages of Oath or Declaration by the applicant(s):
 - a. Newly executed original
 - b. Copy from a prior application (37 CFR 1.63(d)) (for continuation/divisional applns. only)
 - 5 pages of Assignment; and Recordation Cover Sheet
 - 1 page of Sequence Listing; and Attorney's sequence statement.
 - 2 pages of Information Disclosure Statement; and PTO Form 1449 (3 pgs.)
2. The filing fee is calculated below:

For	Number Filed	Number Extra	Rate	Fee
Total Claims	12	- 20 = 0	x \$18.00 =	\$ 0.00
Independent Claims	4	- 3 = 1	x \$80.00 =	80.00
Multiple Dependent Claims	0		+ \$270.00 =	0.00
Basic Fee			\$710.00 =	710.00
			Total Filing Fee	\$ 790.00

3. Please charge Deposit Account No. 01-0519, in the name of Amgen Inc., in the amount of \$ 790.00. An original and one copy are enclosed.
4. Throughout the prosecution of this application, if any extension of time is necessary, please consider this a request therefor.
5. The Commissioner is hereby authorized to charge any additional filing fees which may be required by the accompanying application, any additional fees which may be required during pendency of this application as required by 37 CFR 1.16 or 1.17, or credit any overpayment to Deposit Account No. 01-0519 throughout the prosecution of this application.
6. Cancel in this application original claims of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

EXPRESS MAIL CERTIFICATE

"Express Mail" mailing number

EL360687267US

Date of Deposit:

November 28, 2000

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Box Patent Application, Assistant Commissioner for Patents, Washington, D.C. 20231

lynne Buchsbaum

Printed Name

lynne Buchsbaum

Signature

7. Preliminarily, please amend the specification by inserting before the first line the following:

--This application is a continuation in part of application Serial No. 09/221,181, filed December 23, 1998, which is hereby incorporated by reference.--

8. Transfer the drawings from the prior application to this application and abandon said prior application as of the filing date accorded this application. A duplicate copy of this sheet is enclosed for filing in the prior application file. (May only be used if signed by person authorized by § 1.138 and before payment of base issue fee.)

8a. New formal drawings are enclosed.

9. Priority of application Serial No. _____ filed on _____ in _____
is claimed under 35 USC 119. _____ (country)

9a. The certified copy has been filed in prior application Serial No. _____ filed _____

10. The prior application is assigned of record to Amgen Inc.

11. A preliminary amendment is enclosed.

12. Also enclosed _____

13. Other: _____

14. The power of attorney in the prior application is to:

Ron K. Levy, Registration No.: 31,539

Steven M. Odre, Registration No.: 29,094

Craig A. Crandall, Registration No.: 38,416

- a. The power appears in the original papers in the prior application.
- b. Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.
- c. Address all future communications to
Craig A. Crandall
at the address below.

Signator: Assignee of complete interest

Attorney or agent of record



Craig A. Crandall
Craig A. Crandall
Attorney/Agent for Applicant(s)
Registration No. 38,416
Phone: (805) 447-3011
Date: November 28, 2000

Please send all future correspondence to:

U. S. Patent Operations/ CAC
Dept. 4300, M/S 27-4-A
AMGEN, INC.
One Amgen Center Drive
Thousand Oaks, California 91320-1799

POLYOL/OIL SUSPENSIONS FOR THE SUSTAINED
RELEASE OF PROTEINS

This application is a continuation in part of
5 U.S. patent application serial no. 09/221,181, filed
December 23, 1998 which is incorporated by reference
herein.

BACKGROUND OF THE INVENTION

10

Due to recent advances in genetic and cell engineering technologies, proteins known to exhibit various pharmacological actions *in vivo* are capable of being produced in large amounts for pharmaceutical applications. Such pharmaceutical proteins include erythropoietin (EPO), novel erythropoiesis stimulating protein (NESP), granulocyte colony-stimulating factor (G-CSF), interferons (alpha, beta, gamma, consensus), tumor necrosis factor binding protein (TNFbp), interleukin-1 receptor antagonist (IL-1ra), brain-derived neurotrophic factor (BDNF), keratinocyte growth factor (KGF), stem cell factor (SCF), megakaryocyte growth differentiation factor (MGDF), osteoprotegerin (OPG), glial cell line derived neurotrophic factor (GDNF), somatotrophins and obesity protein (OB protein). OB protein may also be referred to herein as leptin.

Many illnesses or conditions treated with pharmaceutical proteins require sustained protein levels to achieve the most effective therapeutic result. However, as with most protein pharmaceuticals, the generally short biological half-life requires

frequent administration. These repeated injections are given at various intervals which result in fluctuating medication levels at a significant physical and monetary burden on the patients. Since many conditions

5 respond better to controlled levels of a pharmaceutical, a need exists for controlled release of a medicament to provide longer periods of consistent release. Such sustained-release medicaments would provide a means of controlling blood levels of the

10 active ingredient, thus providing the patient with enhanced prophylactic, therapeutic or diagnostic effects, as well as greater safety, patient convenience and patient compliance. Also such sustained release compositions can lead to dose sparing and thus lower

15 cost of protein production. Unfortunately, the instability of most proteins (e.g. denaturation and loss of bioactivity upon exposure to heat, organic solvents, etc.) has greatly limited the development and evaluation of sustained-release formulations.

20 Attempts to develop sustained-release formulations have included the use of a variety of biodegradable and non-biodegradable polymer (e.g. poly(lactide-co-glycolide)) microparticles containing the active ingredient (see e.g., Wise et al.,

25 Contraception, 8:227-234 (1973); and Hutchinson et al., Biochem. Soc. Trans., 13:520-523 (1985)), and a variety of techniques are known by which active agents, e.g. proteins, can be incorporated into polymeric microspheres (see e.g., U.S. Patent No. 4,675,189 and

30 references cited therein). Unfortunately, some of the sustained release devices utilizing microparticles still suffer from such things as: low entrapment efficiency; active agent aggregation formation; high

PAPERS - 4552262

initial bursts of active agent with minimal release thereafter; and incomplete release of active agent.

- Other drug-loaded polymeric devices have also been investigated for long term, therapeutic treatment
5 of various diseases, again with much attention being directed to polymers derived from alpha hydroxycarboxylic acids, especially lactic acid in both its racemic and optically active form, and glycolic acid, and copolymers thereof. These polymers are
10 commercially available and have been utilized in FDA-approved systems, e.g., the Lupron Depot™, which consists of injectable microparticles which release leuprolide acetate for about 30 days for the treatment of prostate cancer.
15 Various problems identified with the use of such polymers include: inability of certain macromolecules to diffuse out through the matrix; deterioration and decomposition of the drug (e.g., denaturation caused by the use of organic solvents);
20 irritation to the organism (e.g. side effects due to use of organic solvents); low biodegradability (such as that which occurs with polycondensation of a polymer with a multifunctional alcohol or multifunctional carboxylic acid, i.e., ointments); and slow rates of
25 degradation.

- A variety of oil based formulations have been described. Welch in U.S. Patent No. 2,491,537 discloses the use of oil suspensions (gelled vegetable oil) to provide 24 hour release of penicillin.
30 Buckwalter in U.S. Patent No. 2,507,193 discloses release in rabbits for up to eleven days using procaine penicillin suspended in peanut oil gelled with 5% aluminum monostearate (AIMS). Anschel in U.S. Patent

No. 2,964,448 discloses suspensions of relaxin in a vegetable oil gelled with AIMS. Anschel reports 5-7 days of relaxation and discloses longer effect (up to 23 days) by heat treating the suspension containing
5 AIMS. Yamahira et al. in U.S. Patent No. 4,855,134 disclose sustained-release preparations of indomethacin or interferon in admixture with a pharmaceutically acceptable biodegradable carrier, e.g., gelatin. Mitchell in U.S. 5,411,951 discloses compositions
10 wherein metal-associated somatotropin is present in a biocompatible oil and it is demonstrated that the compositions can be parenterally administered for prolonged release of somatotropin in animals. Ferguson et al. in U.S. 4,977,140 disclose sustained release
15 formulations comprising bovine somatotropin, a wax, and an oil. Reichert et al. in WO 96/18417 disclose pharmaceutical compositions comprising mixtures of crystalline G-CSF and vegetable oils.

There have also been a number of reports
20 discussing efforts to develop drug delivery systems utilizing protein that are subject to aggregation. For example, Grodsky et al., U.S. Patent No. 4,371,523, describe the use of anti-aggregation agents, e.g., glutamic acid and/or aspartic acid, to develop insulin
25 formulations. Blackshear et al., U.S. Patent 4,439,181, describe mixing glycerol or another polyol with an aqueous protein hormone solution prior to the introduction of the solution into the drug delivery system. Wigness et al., PCT Publication WO 85/02118
30 describe the use of glycerol to prevent precipitation of proteins within drug delivery systems; and Azain et al., EP Publication 0 374 120 A2 describe stable

somatotropin compositions which utilize, *inter alia*, a stabilizing polyol.

Despite the advances made in the processes described above, there is still a need to develop 5 pharmaceutical formulations which achieve a more versatile and effective means of sustained-release for clinical applications. Numerous recombinant or natural proteins could benefit from constant long term release and thereby provide more effective clinical results.

10 Human recombinant G-CSF selectively stimulates neutrophils, a type of white blood cell used for fighting infection. Currently, Filgrastim®, a recombinant G-CSF, is available for therapeutic use. The structure of G-CSF under various conditions has 15 been extensively studied; Lu et al., *J. Biol. Chem.* Vol. 267, 8770-8777 (1992).

G-CSF is labile and highly susceptible to environmental factors such as temperature, humidity, oxygen and ultraviolet rays. And, because of its 20 hydrophobic characteristics, G-CSF is difficult to formulate due to formation of dimer and higher order aggregates (macro range) during long-term storage. G-CSF has been shown to be very prone to aggregation, especially at neutral pH, elevated salt and 25 temperatures (i.e. physiological serum conditions). This instability makes the sustained release (of a period of one week or greater) by conventional delivery systems very problematic, and in fact, such systems generally provide only a few days of release at best.

30 It is an object of the present invention to produce a G-CSF-containing preparation which would provide for the sustained release of G-CSF. Production of such preparations is achieved using glycerol/oil

suspensions containing G-CSF, and, importantly, pharmaceutical compositions using these G-CSF/glycerol/oil suspensions are capable of providing increased bioavailability, protein protection,
5 decreased degradation and slow release with increased protein stability and potency. Importantly, pharmaceutical compositions of the present invention provide a simple, rapid and inexpensive means of controlled recombinant protein release for effective
10 prophylactic, therapeutic or diagnostic results.

SUMMARY OF THE INVENTION

The present invention thus relates to the
15 preparation of a stabilized, prolonged-release injectable suspension containing a biologically active agent. The present invention stems from the observation that G-CSF is stabilized when admixed in glycerol and remains stabilized when the mixture is
20 further suspended in a thickened oil such as sesame oil containing a low percentage of aluminum monostearate, or wax, thus providing a stabilized, prolonged-release injectable preparation. Surprisingly, and unexpectedly, it has now been determined that G-CSF in
25 solution is soluble in high concentrations of glycerol, and still provides a stabilized, prolonged-release injectable preparation when incorporated into a thickened oil.

In one embodiment, the present invention
30 provides sustained-release suspensions comprising an effective amount of a biologically active agent (BAA) incorporated into a polyol/thickened oil suspension, said suspension capable of providing for the sustained-

release of the biologically active agent for a period of at least one week.

In another embodiment, the present invention provides a method for the parenteral administration of
5 a BAA/glycerol/oil suspension to a warm blooded animal, wherein said suspension is administered subcutaneously, or intramuscularly and the biologically active agent is released from the suspension at a controlled rate for up to one week or more.

10 The present invention further relates to pharmaceutical compositions comprising the sustained release suspensions together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, anti-oxidants (e.g., ascorbic acid and
15 Vitamin E), adjuvants and/or carriers needed for administration.

The present invention further relates to processes for preparing sustained-release injectable BAA/polyol/oil suspensions as above. The principal
20 embodiment comprises: (a) admixing a BAA in a polyol to form a BAA/polyol mixture and (b) suspending said BAA/polyol mixture in a mixture comprising a thickened oil, or wax, to form a BAA/polyol/oil suspension.

The present invention further relates to a
25 prefilled syringe comprising said formulation.

The present invention also relates to methods of treatment of individuals using the stabilized, prolonged-release injectable preparations described herein.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the following terms shall have the following meaning:

5 "Biodegradable" is defined as meaning that the polyol/oil vehicle will erode or degrade or absorb or metabolize *in vivo* to form smaller non-toxic components.

10 "Biocompatible" is defined as meaning the oil and its thickeners and other excipients will have no intolerable adverse effect on the polypeptide, or human being treated.

15 "Parenteral administration" is defined as meaning any route of administration other than the alimentary canal, including, for example, subcutaneous, intramuscular, intrathecal, intraorbital, intraarticular, pulmonary, nasal, rectal and otic.

20 As used herein, biologically active agents refers to recombinant or naturally occurring proteins, whether human or animal, useful for prophylactic, therapeutic or diagnostic application. The biologically active agent can be natural, synthetic, semi-synthetic or derivatives thereof. In addition, biologically active agents of the present invention can 25 be PEGylated or conjugated with water soluble adducts such as carbohydrates, e.g., dextran. A wide range of biologically active agents are contemplated. These include but are not limited to hormones, cytokines, hematopoietic factors, growth factors, antiobesity factors, trophic factors, anti-inflammatory factors, 30 and enzymes (see also U.S. Patent No. 4,695,463 for additional examples of useful biologically active agents). One skilled in the art will readily be able

to adapt a desired biologically active agent to the compositions of present invention which can also include small organic or organometallic compounds.

Such proteins would include but are not

- 5 limited to granulocyte-colony stimulating factors (G-CSF's) (see, U.S. Patent Nos. 4,810,643, 4,999,291, 5,581,476, 5,582,823, and PCT Publication No. 94/17185, hereby incorporated by reference including drawings), interferons (see, U.S. Patent Nos. 5,372,808, 5,541,293
- 10 4,897,471, and 4,695,623 hereby incorporated by reference including drawings), interleukins (see, U.S. Patent No. 5,075,222, hereby incorporated by reference including drawings), erythropoietins (see, U.S. Patent Nos. 4,703,008, 5,441,868, 5,618,698 5,547,933, and
- 15 5,621,080 hereby incorporated by reference including drawings), stem cell factor (PCT Publication Nos. 91/05795, 92/17505 and 95/17206, hereby incorporated by reference including drawings), osteoprotegerin (PCT Publication No. 97/23614, hereby incorporated by
- 20 reference including drawings), novel erythropoiesis stimulating protein (NESP) (PCT Publication No. 94/09257, hereby incorporated by reference including drawings) and leptin (OB protein).

Provided below is a working example using

- 25 G-CSF, which, as described above, is a therapeutic protein used to treat hematopoietic disorders. In general, G-CSF useful in the practice of this invention may be a form isolated from mammalian organisms or, alternatively, a product of chemical synthetic procedures or of prokaryotic or eukaryotic host expression of exogenous DNA sequences obtained by genomic or cDNA cloning or by DNA synthesis. Suitable prokaryotic hosts include various bacteria (e.g.,

SEARCHED
INDEXED
MAILED
FILED

E. coli); suitable eukaryotic hosts include yeast (e.g., *S. cerevisiae*) and mammalian cells (e.g., Chinese hamster ovary cells, monkey cells). Depending upon the host employed, the G-CSF expression product 5 may be glycosylated with mammalian or other eukaryotic carbohydrates, or it may be non-glycosylated. The G-CSF expression product may also include an initial methionine amino acid residue (at position -1). The present invention contemplates the use of any and all 10 such forms of G-CSF, although recombinant G-CSF, especially *E. coli* derived, is preferred, for, among other things, greatest commercial practicality.

Certain G-CSF analogs have been reported to be biologically functional, and these may also be 15 chemically modified, by, for example, the addition of one or more polyethylene glycol molecules. G-CSF analogs are reported in U.S. Patent No. 4,810,643. Examples of other G-CSF analogs which have been reported to have biological activity are those set forth in AU-A-76380/91, EP O 459 630, EP O 272 703, 20 EP O 473 268 and EP O 335 423, although no representation is made with regard to the activity of each analog reportedly disclosed. See also AU-A-10948/92, PCT 94/00913 and EP O 243 153. Of course, if one so desires when treating non-human 25 mammals, one may use recombinant non-human G-CSF's, such as recombinant murine, bovine, canine, etc. See PCT WO 9105798 and PCT WO 8910932, for example.

The type of G-CSF used for the present 30 preparations may be selected from those described in PCT Publication No. 94/17185, as cited above and herein incorporated by reference in its entirety. The 174 amino acid sequence for mature, recombinant methionyl

human G-CSF is presented herein as SEQ ID NO: 1, where the first amino acid of the mature protein is threonine (T) (at position 1) and a methionyl residue is located at position -1 (not included in the sequence below).

5

SEQ ID NO: 1

	T	P	L	G	P	A	S	S	L	P	Q	S	F	L
10	L	K	C	L	E	Q	V	R	K	I	Q	G	D	G
	A	L	Q	E	K	L	C	A	T	Y	K	L	C	H
	E	E	L	V	L	L	G	H	S	L	G	I	P	W
	P	L	S	S	C	P	S	Q	A	L	Q	L	A	G
	L	S	Q	L	H	S	G	L	F	L	Y	Q	G	L
15	Q	A	L	E	G	I	S	P	E	L	G	P	T	L
	T	L	Q	L	D	V	A	D	F	A	T	T	I	W
	Q	M	E	E	L	G	M	A	P	A	L	Q	P	T
	G	A	M	P	A	F	A	S	A	F	Q	R	R	A
	G	V	L	V	A	S	H	L	Q	S	F	L	E	V
20	Y	R	V	L	R	H	L	A	Q	P				S

However, as with any of the present G-CSF moieties, the methionyl residue at position -1 may be absent.

Also included are those proteins as set forth above with amino acid substitutions which are "conservative" according to acidity, charge, hydrophobicity, polarity, size or any other characteristic known to those skilled in the art. These are set forth in Table 1, below. See generally, Creighton, *Proteins, passim* (W.H. Freeman and Company, N.Y., 1984); Ford et al., *Protein Expression and Purification* 2:95-107 (1991), which are herein incorporated by reference.

Table 1

5

Basic:	arginine lysine histidine
Acidic:	glutamic acid aspartic acid
Polar:	glutamine asparagine
Hydrophobic:	leucine isoleucine valine
Aromatic:	phenylalanine tryptophan tyrosine
Small:	glycine alanine serine threonine methionine

In addition, biologically active agents can also include but are not limited to insulin, gastrin, prolactin, adrenocorticotrophic hormone (ACTH), thyroid stimulating hormone (TSH), luteinizing hormone (LH), follicle stimulating hormone (FSH), human chorionic gonadotropin (HCG), motilin, interferons (alpha, beta, gamma), interleukins (IL-1 to IL-12), tumor necrosis factor (TNF), tumor necrosis factor-binding protein (TNF-bp), brain derived neurotrophic factor (BDNF),

glial derived neurotrophic factor (GDNF), neurotrophic factor 3 (NT3), fibroblast growth factors (FGF), neurotrophic growth factor (NGF), insulin-like growth factors (IGFs), macrophage colony stimulating factor 5 (M-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), megakaryocyte derived growth factor (MGDF), keratinocyte growth factor (KGF), thrombopoietin, platelet-derived growth factor (PGDF), colony simulating growth factors (CSFs), bone 10 morphogenic protein (BMP), superoxide dismutase (SOD), tissue plasminogen activator (TPA), urokinase, somatotropins, streptokinase and kallikrein. The term proteins, as used herein, includes peptides, polypeptides, consensus molecules, analogs, derivatives 15 or combinations thereof.

The BAA used to prepare the sustained-release compositions of the present invention can be in solution or powder form and is first admixed with a polyol, e.g., glycerol. Precise concentrations of 20 polyol will be used, depending upon the amount of BAA used. The polyol is added in an amount sufficient to stabilize (e.g., prevent aggregation) the BAA during long-term storage of the BAA in the suspension.

Other biocompatible C-4 to C-19 polyols 25 contemplated for use include, but are not limited to, C-4: erythritol; C-5: arabinose, xylose, ribose; C-6: inositol, fructose, galactose, glucose, mannose; C-12: maltose and sucrose. If the polyol used is in solid form, it will be first prepared as an aqueous or 30 aqueous organic solution or fluidized by means of heat or pressure, and admixed with the BAA.

The level of polyol used to prepare the BAA/polyol mixture can range from 5%-100% (e.g., 100% =

neat glycerol; 90% = 90% glycerol, 10% water). When the BAA to be used is in powdered form, the resultant BAA/polyol mixture will be in the form of a suspension. When the BAA to be used is in solution form, the
5 resultant BAA/polyol mixture will be in solution form. The polyol concentration in the final BAA/polyol/oil suspension can range from 10%-40%, more preferably 20%-30%. In a preferred embodiment wherein G-CSF powder is the biologically active agent, and glycerol is the
10 polyol, 20% glycerol is used in the final suspension.

The oils used in the present invention are biocompatible, of low acidity and essentially free from rancidity. Such oils are selected from the group consisting of, for example, sesame seed, canola, saffron, castor, cottonseed, olive, peanut, sunflower
15 seed, ethyl oleate, vitamin E including α -tocopherol and its derivatives, and Miglyol 812.

The glycerol/oil suspensions will also contain a "thickener" or "gelling agent" which serves
20 to retard hydration of the suspension, give the body of oil greater viscosity or viscoelasticity, and thereby decrease the rate of release of the BAA from the suspension following administration and also increase the stabilization of the BAA, and increase the physical
25 stability of the suspension as a whole (i.e., prevent phase separation). Such agents include polyvalent metal salts of organic acids, e.g., aluminum, zinc, magnesium or calcium salts of lauric acid, palmitic acid, stearic acid and the like, and oleaginous
30 materials such as waxes and high viscosity oils and organic or inorganic fillers such as polymers and salts. Aluminum monostearate and distearate and white wax are particularly preferred agents. Said agents are

usually present at concentrations (based on weight of oil) of between about 0.1% and about 99%, more typically between about 0.5% and about 90% and for metal salts even more typically 0.5% to 20%. This 5 ratio is important for purposes of assuring that the agent doesn't increase the viscosity of the suspension to the point where the suspension is no longer useful for injection through a syringe. For highly viscous formulations, implants are also contemplated.

10 The glycerol/oil suspensions may further comprise surface active agents or emulsifiers to stabilize the glycerol/oil suspension and prevent it from separating. This surface active agent or emulsifier can be ionic or nonionic and may be selected 15 from the group consisting of, for example, Span 40, Span 80, Pluronics®, and egg lecithin, or mixtures thereof, preferably with a HLB (hydrophile-lipophile balance) of 1-10, more preferably 2-8, and even more preferably 4-8. The surfactant can also help dissipate 20 the oil in the biological environment. The surfactant is usually present at 0.1% to 50%, preferably 0.2% to 20%, and more preferably 0.5% to 10% by weight of oil. Certain materials, such as hydrogenated vegetable oil can function as both a thickener and stabilizer of the 25 glycerol suspension.

The BAA/glycerol/oil suspensions of the present invention can be prepared by suspending a biologically active agent (in powdered form) in a substantially pure glycerol solution (at high glycerol 30 concentrations, e.g., 70%-100%) to form a BAA/glycerol suspension, and then suspending said BAA/glycerol suspension in a solution comprising oil alone or oil containing a "gelling agent" suspended or dissolved in

the oil. The oil (containing gelling agent) may first need to be heated (with mixing) to assure that the gelling agent completely dissolves in the oil.

- Alternatively, the BAA/glycerol/oil
- 5 suspensions of the present invention may also be prepared by mixing a biologically active agent (in solution form) in a substantially pure glycerol solution (at high glycerol concentrations, e.g., 70%-100%) to form a BAA/glycerol solution, and then suspending said
- 10 BAA/glycerol solution in a solution comprising oil alone or oil containing a "gelling agent" suspended or dissolved in the oil.

In general, comprehended by the invention are pharmaceutical compositions comprising effective amounts of biologically active agent, or derivative products (e.g., precipitates), together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, anti-oxidants (e.g., ascorbic acid and Vitamin E), adjuvants and/or carriers needed for administration. (See PCT 97/01331 hereby incorporated by reference.) The optimal pharmaceutical formulation for a desired biologically active agent will be determined by one skilled in the art depending upon the route of administration, desired dosage and duration of release. Exemplary pharmaceutical compositions are disclosed in Remington's Pharmaceutical Sciences (Mack Publishing Co., 18th Ed., Easton, PA, pgs. 1435-1712 (1990)). The pharmaceutical compositions of the present invention are particularly attractive for parenteral administration, e.g., by injection intramuscularly, subcutaneously, or intraperitoneally.

008277-15582769

- Therapeutic uses of the compositions of the present invention depend on the biologically active agent used. One skilled in the art will readily be able to adapt a desired biologically active agent to 5 the present invention for its intended therapeutic uses. Therapeutic uses for such agents are set forth in greater detail in the following publications hereby incorporated by reference including drawings.
- Therapeutic uses include but are not limited to uses 10 for proteins like granulocyte-colony stimulating factors (see, U.S. Patent Nos. 4,999,291, 5,581,476, 5,582,823, 4,810,643 and PCT Publication No. 94/17185, hereby incorporated by reference including drawings), interferons (see, U.S. Patent Nos. 5,372,808, 15 5,541,293, hereby incorporated by reference including drawings), interleukins (see, U.S. Patent No. 5,075,222, hereby incorporated by reference including drawings), erythropoietins (see, U.S. Patent Nos. 4,703,008, 5,441,868, 5,618,698 5,547,933, and 20 5,621,080 hereby incorporated by reference including drawings), stem cell factor (PCT Publication Nos. 91/05795, 92/17505 and 95/17206, hereby incorporated by reference including drawings), OB protein (see PCT publication Nos. 96/40912, 96/05309, 97/00128, 25 97/01010 and 97/06816 hereby incorporated by reference including figures), novel erythropoiesis stimulating protein (PCT Publication No. 94/09257, hereby incorporated by reference including drawings), and small molecule drugs. In addition, the present 30 compositions may also be used for manufacture of one or more medicaments for treatment or amelioration of the conditions the biologically active agent is intended to treat.

As specifically relates to G-CSF, the therapeutic has been shown to be effective in treating inflammatory bowel disease. For example, it has been reported that an adolescent boy with Crohn's disease
5 and enterocutaneous fistulas had a response to treatment with G-CSF (filgrastim) after all standard treatments failed; Vaughn and Drumm, *New England Journal of Medicine*, 340(3):239-240 (1999). It has also been reported that prolonged high-dose therapy
10 with G-CSF may have anti-inflammatory effects in colitis; Hommes et al., *Clin Exp. Immunol.*, 106:529-533 (1996). It is thus envisioned that the G-CSF-containing suspensions of the present invention will also be effective in treatment of inflammatory bowel
15 diseases.

One skilled in the art will be able to ascertain effective dosages by administration and observing the desired therapeutic effect. Preferably, for G-CSF, the formulation of the suspension will be
20 such that between about 0.01 µg G-CSF moiety/kg body weight/day and 10 mg G-CSF moiety/kg body weight/day will yield the desired therapeutic effect. The effective dosages may be determined using diagnostic tools over time. For example, a diagnostic for
25 measuring the amount of G-CSF in the blood (or plasma or serum) may first be used to determine endogenous levels of G-CSF protein. Such diagnostic tool may be in the form of an antibody assay, such as an antibody sandwich assay. The amount of endogenous G-CSF protein
30 is quantified initially, and a baseline is determined. The therapeutic dosages are determined as the quantification of endogenous and exogenous G-CSF protein moiety (that is, protein, analog or derivative

002717-10022600

found within the body, either self-produced or administered) is continued over the course of therapy. The dosages may therefore vary over the course of therapy, with, for example, a relatively high dosage 5 being used initially, until therapeutic benefit is seen, and lower dosages used to maintain the therapeutic benefits. Alternatively, the levels of neutrophils are determined and monitored over the course of the therapy. The dosage is adjusted to 10 maintain the required level of neutrophil counts with the lowest frequency of injections.

The following examples are offered to more fully illustrate the invention, but are not to be construed as limiting the scope thereof.

15

EXAMPLE 1

This example describes the preparation of G-CSF powder by spray-drying.

20 G-CSF solution (~2.75 mg/ml, with 5% sorbitol, in 0.58mM HCl) was placed in dialysis tubing (Spectrum Lab Inc., flat width 18 ± 2 mm, diameter 11.5 mm, 1.0 ml/cm), and dialyzed against water (pH 3.25) at 4°C for 24 hours. During the dialysis, the 25 water is changed four times. Dialyzed G-CSF solution (~1100 ml) was then placed in an ultrafiltration cell and air pressure applied on the solution. After two hours, about 300 ml of concentrated G-CSF solution was collected and filtered through a 0.2 mm filter unit.
The concentration of the final G-CSF solution is 30 9.134 mg/ml. The spray-drying was performed on a BUCHI 190 Mini Spray Dryer (Brinkmann Institute), and all of the glassware of the spray dryer was first washed with

deionized water, followed by sterile water, followed by ethanol. The spray-drying was performed with inlet air flow of 450 normal liters/hour, and the feed rate of G-CSF solution was 1.0 mL/min. G-CSF powder (2.640
5 grams, 82.7% G-CSF) was obtained from the 290 mL starting G-CSF solution.

EXAMPLE 2

10 This example describes the preparation of G-CSF/glycerol suspensions and the use of the G-CSF/glycerol suspensions to prepare G-CSF/glycerol/oil formulations.

Step 1. A G-CSF/glycerol suspension was first prepared by placing 105.4 milligrams G-CSF spray-dried powder (prepared as described in Example 1) and 2.401 mL neat glycerol in a mortar and grinding the mixture until no coarse particles were seen.

Step 2. A thickened oil suspension was then prepared by placing 45.67 grams sesame oil (Croda, Inc.) and 1.91 grams aluminum monostearate (AIMS) (Fluka) in a 125 mL erlenmeyer flask and mixing with a magnetic stirrer at room temperature for 20 minutes, followed by heating at 165°C-170°C under nitrogen atmosphere with stirring. The stirring is continued for two hours, and the mixture then cooled to room temperature, resulting in an opalescent gel-like thickened oil (3% AIMS).

Step 3. One mL G-CSF/glycerol suspension and 4 mL thickened oil were placed in a mortar and ground together until well mixed. The suspension (G-CSF/20% glycerol/3% AIMS/oil) was stored in a sterile sample vial at 4°C until needed.

EXAMPLE 3

This example describes the preparation of a
5 G-CSF/glycerol-containing viscous oil suspension
further containing L-ascorbic acid and surfactant.

L-Ascorbic acid (50 mg) was dissolved in a
1 mL glycerol solution by heating and stirring the
mixture. After being cooled to room temperature, the
10 ascorbic acid/glycerol solution was mixed with GCSF
powder (45.3 mg) and Span 80 (250 mL).

3.75 mL thickened oil (3% AIMS) prepared as
described above was added to the G-CSF/ascorbic acid/
glycerol mixture and ground together to give a viscous
15 oil suspension (G-CSF/20% glycerol+ascorbic acid/Span
80/3% AIMS/oil).

EXAMPLE 4

20 This example shows the preparation of an oil
thickened with 7% white wax.

The thickened 7% wax/oil was produced (using
the procedure described in Example 2, Step 2) by
heating a mixture of white wax (4.49 grams) and sesame
25 oil (59.65 grams) at 160°C under nitrogen atmosphere
for 2 hours.

EXAMPLE 5

30 This example shows the preparation of various
G-CSF-containing oil formulations using 7% wax as
thickener and with different glycerol levels.

Preparation 1: G-CSF powder (27.6 mg) and glycerol (600 μ L) were mixed in a mortar and ground until no observable coarse particles were seen. Then 2.4 mL of the thickened 7% wax/oil prepared as

5 described in Example 4 was added to the GCSF/glycerol suspension. The mixture was ground together with mortar and pestle to give a viscous oil formulation (G-CSF/20% glycerol/7% wax).

Preparation 2: GCSF powder (45.3 mg) was
10 mixed with 1.00 ml of ascorbic acid/glycerol solution (prepared as described in Example 3), and then 4.0 mL of thickened 7% wax/oil was added. The resulting mixture was ground together to give a viscous oil formulation (G-CSF/20% glycerol+ascorbic acid/7% wax).

15 Preparation 3: G-CSF powder (27.3 mg) and glycerol (450 μ L) were mixed in a mortar and ground until no observable coarse particles were seen. Then 2.55 mL of the thickened 7% wax/oil prepared as described in Example 4 was added to the GCSF/glycerol
20 suspension. The mixture was ground together with mortar and pestle to give a viscous oil formulation (G-CSF/15% glycerol/7% wax).

Preparation 4: G-CSF powder (27.5 mg) and glycerol (750 μ L) were mixed in a mortar and ground
25 until no observable coarse particles were seen. Then 2.25 mL of the thickened 7% wax/oil prepared as described in Example 4 was added to the GCSF/glycerol suspension. The mixture was ground together with mortar and pestle to give a viscous oil formulation (G-CSF/25% glycerol/7% wax).

123456789101112131415161718191A

EXAMPLE 6

This example shows the preparation of an
5 G-CSF/glycerol oil thickened with 10% white wax.

The thickened 10% wax/oil was produced (using
the procedure described in Example 2, Step 2) by
heating a mixture of white wax (6.5 grams) and sesame
oil (58.5 grams) at 160°C under nitrogen atmosphere for
10 2 hours.

GCSF powder (27.4 mg) and neat glycerol (600
μl) were mixed together, and then 2.40 mL of thickened
oil (10% wax) was added to the GCSF/glycerol
suspension. The mixture was ground to give a viscous
15 oil formulation (G-CSF/20% glycerol/10% wax).

EXAMPLE 7

This example describes the *in vivo* testing of
20 the suspensions prepared in Examples 2-6.

Splenectomized mice (BDF1) were injected once
(subcutaneously) with 30 mg/kg of the various
G-CSF-containing suspensions, and the various controls.
The mice had their blood analyzed over several days.
25 G-CSF powder (- glycerol) in 3% AIMS oil (30 mg/kg);
G-CSF powder in glycerol (30 mg/kg); G-CSF powder
dissolved in water (30 mg/kg); and 1X PBS were run as
controls. The data is summarized in Table 1 below.

Table 1

	<u>Formulation</u>	<u>Neutrophil Count ($10^6/\text{mL}$)</u>		
		<u>Day 3</u>	<u>Day 5</u>	<u>Day 7</u>
5	1X PBS	2.0	2.0	2.0
10	G-CSF in pH 3.25 water (+ 5% sorbitol)	2.0	2.0	2.0
15	G-CSF in glycerol	3.5	2.0	2.0
20	G-CSF (no glycerol) in 3% AIMS/oil	1.5	1.5	1.5
25	G-CSF/20% glycerol 3% AIMS/oil	24	33	19
30	G-CSF/20% glycerol ascorbic acid/Span 80 3% AIMS/oil	18.1	23.8	8.7
35	G-CSF/20% glycerol 7% wax/oil	27	40.2	10.3
40	G-CSF/15% glycerol 7% wax/oil	32.4	36	8.1
	G-CSF/25% glycerol 7% wax/oil	24.6	38.2	13.9
	G-CSF/20% glycerol 10% wax/oil	33.6	56.9	25.6

As evidenced by the data in Table 1, the polyol/thickened oil suspensions are capable of providing for the sustained release of G-CSF for periods of at least one week. Importantly, it should be noted that G-CSF could not be delivered in the oils without the addition of the polyol.

EXAMPLE 8

This example shows the preparation of an oils thickened with glycerin stearate.

Preparation 1: Glycerol tristearate (1.00 gram), glycerol monostearate (4.00 grams), and sesame oil (45.00 grams) were placed in a bottle and heated at

160°C under nitrogen atmosphere for 2 hours. The mixture was then cooled to room temperature while being vortexed. A white thickened oil was obtained.

Preparation 2: Glycerol monostearate (0.80
5 grams) and sesame oil (9.20 grams) were placed in a bottle and heated at 160°C under nitrogen atmosphere for 2 hours. The mixture was then cooled to room temperature while being vortexed. A white thickened oil was obtained.

10

EXAMPLE 9

This example describes the preparation of thick oil using a mixture of sesame oil and the more 15 viscous hydrogenated vegetable oil.

Sesame oil (6.00 mL) and hydrogenated vegetable oil (34.00 mL) were placed in a bottle and the mixture heated at 160°C under nitrogen atmosphere for 2 hours. After the mixture cooled to room 20 temperature, a thickened oil was obtained.

EXAMPLE 10

This example shows the preparation of 25 G-CSF/glycerol in oil suspensions where the oil contains a mixture of sesame and hydrogenated vegetable oil and where the hydrogenated vegetable oil thickens the mixture.

Preparation 1: GCSF powder (10.0 mg) and 30 neat glycerol (0.20 mL) were mixed, and then an oil mixture (hydrogenated oil/sesame oil = 5/3, 0.80 mL) was added. The mixture was ground together with a mortar and pestle to give a viscous suspension

formulation. This formulation was filled into a syringe and was syringable.

Preparation 2: GCSF powder (10.3 mg) and glycerol (0.20 mL) were mixed, and then an oil mixture (hydrogenated oil/sesame oil = 3/17, 0.8 mL) was added. The mixture was ground together with a mortar and pestle to give a viscous suspension formulation. This formulation was filled into a syringe and was syringable.

10

EXAMPLE 11

This example shows the preparation of a thickened oils using stearic acid, stearyl alcohol, and combinations thereof, as thickeners + G-CSF/glycerol.

Preparation 1: Stearic acid (1.00 gram) and sesame oil (9.00 grams) were placed in a bottle and the mixture heated at 160°C under nitrogen atmosphere for 2 hours. After cooling to room temperature with shaking
20 the mixture became a viscous thickened oil.

Preparation 2: Stearyl alcohol (1.00 gram) and sesame oil (9.00 grams) were placed in a bottle and the mixture heated at 160°C under a nitrogen atmosphere for 2 hours. After cooling to room temperature with shaking the mixture became a viscous thickened oil.

Preparation 3: Stearyl alcohol (0.50 grams), stearic acid (0.50 grams), and sesame oil (9.00 grams) are placed in a bottle and the mixture heated at 160°C under nitrogen atmosphere for 2 hours. After cooling 30 to room temperature with shaking the mixture became a viscous thickened oil.

Preparation 4: G-CSF powder (9.8 mg) and neat glycerol (0.20 mL) were mixed and then 0.80 mL of

thickened oil (10% stearyl alcohol) was added. The mixture was ground for 10 minutes to give an oil formulation which was filled into a 1 mL syringe and was syringable.

- 5 Preparation 5: G-CSF powder (10.3 mg) and neat glycerol (0.20 mL) were mixed and then 0.80 mL of thickened oil (10% thickener, stearyl alcohol/stearic acid = 3/1) was added. The mixture was ground for 10 minutes to give an oil formulation which was filled
10 into a 1 mL syringe and was syringable.

EXAMPLE 12

- This example shows the preparation of
15 G-CSF/glycerol/oil emulsion formulations wherein G-CSF was first admixed with an aqueous glycerol (50% glycerol/50% water) phase.

The resultant G-CSF/glycerol phase consisted of 12.7 mg/mL G-CSF, 50% glycerol, 1%(w/v) Pluronic F68, 10 mM acetate (pH 4.0) and 0.44 mM HCl. A mixture of 1% Pluronic L101 in corn oil formed the oil phase. A 50:50 and 70:30 mixture of the two phases were homogenized with a Virtis Handishear homogenizer for 45 seconds to form the respective emulsion
25 formulations.

EXAMPLE 13

- This example is prepared in a similar manner
30 to Example 2 except the G-CSF dose is approximately 10 mg/Kg. After a single injection the neutrophils were elevated for at least one week.

EXAMPLE 14

This example describes the preparation of
5 various G-CSF/glycerol/oil suspensions wherein G-CSF
solutions were mixed with various glycerol solutions to
prepare G-CSF/glycerol mixtures that were then
suspended in thickened oils.

Concentrated G-CSF solution (e.g., 200 mg/mL)
10 was prepared by concentrating bulk G-CSF solution using
a Microcon centrifugal filter device. Volumes of neat
glycerol were added to prepare G-CSF/glycerol solutions
of varying concentrations of G-CSF and glycerol.
Excipients such as methionine were also added to a
15 couple of the G-CSF/glycerol solutions. G-CSF/glycerol
/thickened oil formulations were then prepared in a
similar manner to that described in Example 6. A
G-CSF/glycerol/oil emulsion formulation was also
prepared in a similar manner to that described in
20 Example 12 and comprising an aqueous phase (pH 3)
consisting of 15 mg/mL G-CSF, 30 % glycerol and 2.5%
Pluronic F68; and an oil phase consisting of 2%
aluminum monostearate and 2.5% Pluronic L101; and
wherein the ratio of the oil phase to aqueous phase was
25 80%/20% (v/v).

Various formulations were then prepared and
tested *in vivo* as described above. The injections (100
μL) in the splenectomized mice were at 6 mg/kg, except
for the emulsion formulation, which was at 20 mg/kg.

30 The data is summarized in Table 2 below.

Table 2

		Neutrophil Count ($10^6/\text{mL}$)		
		Day 3	Day 5	Day 7
5	Carrier, 1X PBS	1-2	1-2	1-2
	G-CSF in neat glycerol	27.4	17.4	3.1
	G-CSF in 90% glycerol/10% H ₂ O	26.9	44.7	
	G-CSF in 75% glycerol/25% H ₂ O	26.4	33.1	
	G-CSF in 60% glycerol/40% H ₂ O	24.4	32.0	
10	G-CSF in 10% glycerol/90% H ₂ O (Note: 83% oil phase)	similar to carrier		
	G-CSF in 90% glycerol/10% H ₂ O 0.5 mM methionine	~22	~36	~10
	G-CSF in 70% glycerol/30% H ₂ O 1.0 mM methionine	~13.5	~42	~7
15	G-CSF in 30% glycerol Emulsion	similar to vehicle control without G-CSF		

As evidenced from the Table 2 data, G-CSF solution is soluble in high concentrations of glycerol and stabilized such that suspensions which provide sustained release of G-CSF for a period of at least 5 days can be prepared. The addition of methionine as an excipient also seems to provide added stability which appears to allow for lower concentrations of glycerol to be used.

What is claimed is:

1. A sustained-release suspension comprising a biologically active agent (BAA) incorporated into a biocompatible polyol/thickened oil suspension.

5

2. The suspension of Claim 1 wherein said biocompatible polyol is selected from the group consisting of glycerol, erythritol, arabinose, xylose, ribose, inositol, fructose, galactose, maltose, and sucrose.

3. The suspension of Claim 1 wherein said oil is selected from the group consisting of sesame, castor, cottonseed, canola, saffron, olive, peanut, 15 sunflower seed, α -tocopherol, and ethyl oleate; and wherein said oil contains a thickener which is selected from the group consisting of polyvalent metal salts of organic acids, oleaginous materials such as waxes and high viscosity oils, and organic or inorganic fillers 20 such as polymers and salts.

4. The suspension of Claim 3 wherein the thickener is aluminum monostearate.

25 5. The suspension of Claim 3 wherein the
thickener is white wax.

6. The suspension of Claim 1 wherein said suspension further contains methionine.

30

7. The suspension of claim 1, wherein said biologically active agent is a protein selected from the group consisting of interferon consensus.

erythropoietin, granulocyte-colony stimulating factor (GCSF), stem cell factor (SCF), leptin (OB protein), tumor necrosis factor-binding protein (TNF-bp), interleukin-1 receptor antagonist (IL-1ra), brain
5 derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), neurotrophic factor 3 (NT3), osteoprotegerin (OPG), granulocyte macrophage colony stimulating factor (GM-CSF), megakaryocyte derived growth factor (MGDF), keratinocyte growth
10 factor (KGF), thrombopoietin, and novel erythropoiesis stimulating protein (NESP).

8. A process for preparing a
BAA/polyol/thickened oil sustained-release suspension
15 which comprises:

(a) admixing a BAA in a polyol to form a
BAA/polyol mixture;
(b) suspending said BAA/polyol mixture
in a mixture comprising a thickened oil to form a
20 BAA/polyol/oil suspension.

9. A method for the parenteral
administration of a BAA/polyol/oil suspension to a warm
blooded animal, wherein said suspension is administered
25 subcutaneously, or intramuscularly and the biologically
active agent is released from the suspension at a
controlled rate for up to one week or more.

10. A prefilled syringe containing the
30 sustained release suspension of Claim 1.

11. A pharmaceutical composition comprising a sustained-release suspension of Claim 1 and a pharmaceutically acceptable carrier.

5 12. A sustained-release suspension comprising a biologically active agent (BAA) incorporated into a biocompatible polyol/thickened oil suspension, and wherein said suspension is prepared by a process which comprises:

0 (a) admixing a BAA in a polyol to form a BAA/polyol mixture;

 (b) suspending said BAA/polyol mixture in a mixture comprising a thickened oil to form a BAA/polyol/oil suspension.

卷之三

ABSTRACT

The present invention relates to the preparation of polyol/thickened oil suspensions containing a biologically active agent, for the sustained delivery of the biologically active agent. The described protein/glycerol/oil suspensions show sustained release of protein, e.g., G-CSF, of up to at least one week.

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first, and sole inventor (if only one name is listed below) or a joint inventor (if plural names are listed below) of the invention entitled

POLYOL/OIL SUSPENSIONS FOR THE SUSTAINED RELEASE OF PROTEINS

which is described and claimed in the specification which:

- is attached hereto.
 was filed on _____
 as Application Serial No. _____
 and was amended on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS
09/221,181	12/23/1998	Pending

Power of Attorney: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Steven M. Odre (Reg. No. 29,094), Ron K. Levy (Reg. No. 31,539), Scott N. Bernstein (Reg. No. 38,827), Joseph W. Bulock (Reg. No. 37,103), Robert R. Cook (Reg. No. 31,602), Monique L. Cordray (Reg. No. 34,802), Craig A. Crandall (Reg. No. 38,416), Daniel R. Curry (Reg. No. 32,727), Joan D. Eggert (Reg. No. 32,980), Timothy J. Gaul (Reg. No. 33,111), Matthew W. Knight (Reg. No. 36,846), Richard J. Mazza (Reg. No. 27,657), Karen L. Nicastro (Reg. No. 35,968), Nancy A. Oleski (Reg. No. 34,688), Karol M. Pessin (Reg. No. 34,899), Frank S. Ungemach (Reg. No. 34,449), Stuart L. Watt (Reg. No. 32,511), Wendy A. Whiteford (Reg. No. 36,964), Robert B. Winter (Reg. No. 34,458), Thomas D. Zindrick (Reg. No. 32,185), said attorney(s)/agent(s) to have in addition full power of revocation, including the power to revoke any power herein granted.

DECLARATION AND POWER OF ATTORNEY (cont'd)

Please send all future correspondence to:

U.S. Patent Operations/ CAC
Dept. 4300, M/S 27-4-A
AMGEN INC.
One Amgen Center Drive
Thousand Oaks, California 91320-1799

Direct Telephone Calls To:

Craig A. Crandall
Attorney/Agent for Applicant(s)
Registration No.: 38,416
Phone: (805) 447-3011

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Full Name of Sole
or First Inventor:

Merrill Goldenberg

Inventor's Signature:

Date:

Nov 28, 2000

Residence and
Post Office Address:

2616 2616 Radcliffe Rd., Thousand Oaks, CA 91360 USA

(Address, City, State, Zip Code, Country)

Citizenship:

Canadian

Full Name of Second
Joint Inventor, if Any:

Daxian Shan

Inventor's Signature:

Date:

Nov. 28, 2000

Residence and
Post Office Address:

13823 E. Hillcrest Dr., #534, Thousand Oaks, CA 91362 USA

(Address, City, State, Zip Code, Country)

Citizenship:

Peoples Republic of China

Full Name of Third
Joint Inventor, if Any:

Alice Beekman

Inventor's Signature:

Date:

Nov 28, 2000

Residence and
Post Office Address:

300 Rolling Oaks Dr., #184, Thousand Oaks, CA 91361 USA

(Address, City, State, Zip Code, Country)

Citizenship:

United States

DECLARATION AND POWER OF ATTORNEY (cont'd)

Full Name of Fourth
Joint Inventor, if Any:

Tiansheng Li

Inventor's Signature:

 Date: Nov. 28. 2000

Residence and
Post Office Address:

3001 Sunflower St., Thousand Oaks, CA 91360 USA

(Address, City, State, Zip Code, Country)

Citizenship:

United States

DRAFT - 10/2000

SEQUENCE LISTING

<110> GOLDENBERG, MERRILL
SHAN, DAXIAN
BEEKMAN, ALICE

<120> POLYOL/OIL SUSPENSIONS FOR SUSTAINED RELEASE OF
PROTEINS

<130> A-576

<140> NOT ASSIGNED YET
<141> 1998-12-23

<160> 1

<170> PatentIn Ver. 2.0

<210> 1

<211> 174

<212> PRT

<213> granulocyte colony-stimulating factor

<400> 1

Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
1 5 10 15

Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
20 25 30

Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val
35 40 45

Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys
50 55 60

Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser
65 70 75 80

Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser
85 90 95

Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp
100 105 110

Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro
115 120 125

Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe
130 135 140

Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe
145 150 155 160

Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
165 170